

## SPECIFIC DIMER FORMATION OF RIBOSOMAL RNA AND OF VIRAL RNA IN THE PRESENCE OF FORMALDEHYDE

H.SLEGERS and W.FIERS

*Laboratory of Molecular Biology and Laboratory of Physiological Chemistry,  
University of Ghent, Ghent, Belgium*

Received 27 January 1970

### 1. Introduction

More or less specific aggregation effects have been described for ribosomal RNA [1, 2], tRNA [3, 4], viral RNA [5] and synthetic polyribonucleotides [6, 7]. Dimers have been detected both in tRNA [8, 9] and in viral RNA preparations [10], although usually in low amounts.

Bacteriophage MS2 RNA, which normally sediments as 27 S, unfolds to a 13.4 S conformation upon reaction with formaldehyde. We have found, however, that nearly complete conversion to a dimer can be obtained by carrying out the formaldehyde reaction at acidic pH and in the presence of low concentrations of divalent cations [11]. This dimer sediments at approx. 37.5 S, which indicates that it has a compact conformation, analogous to the unreacted 27 S form. Nevertheless, hyperchromicity measurements show that the dimer has reacted to the same extent as the completely unfolded form and that nearly all base pairing has been eliminated. Presumably, the compact dimer conformation is "fixed" by the formation of irreversible methylene bridges between purine bases [12, 13].

In this paper we demonstrate that both the ribosomal RNA species from *Escherichia coli* form dimers, and that the conditions for their formation are exactly the same as for MS2 RNA. Most important, the process is highly sequence specific and no hybrid interaction between 16 S and 23 S RNA was observed.

### 2. Materials and methods

16 S and 23 S rRNA from *E. coli* MRE 600 were

isolated by treatment of ribosomes with phenol in the presence of sodium dodecylsulphate. The two components were separated by centrifugation on a 5 to 20% sucrose gradient in 0.1 M NaCl, 0.01 M sodium acetate and 0.001 M EDTA, pH 5.0. 100 A<sub>260</sub>-units of rRNA was layered on two buckets of the SW-27 rotor and centrifuged for 15 hr at 27,000 rpm and 5°C. The tubes (38.5 ml) were fractionated and the 16 S and 23 S peaks pooled separately. The RNA was precipitated with ethanol and pelleted. This precipitate was further washed twice ethanol and dissolved in  $5 \times 10^{-4}$  M phosphate.

Bacteriophage MS2 RNA was prepared as described previously [11]. The reaction of RNA with formaldehyde was carried out in two steps, as explained in the legends to the figures. The reaction mixtures were analyzed by centrifugation in the Analytical Ultracentrifuge Spinco model E at 20°C and 52,640 rpm. Sedimentation coefficients were calculated from photographs taken with the U.V. optical system at constant time intervals.

### 3. Results

Formaldehyde reactions were carried out in different conditions of pH and NaCl concentrations on a mixture of 16 S and 23 S ribosomal RNA, and the results were compared with those previously obtained with viral RNA [11].

Under appropriate conditions, reaction with formaldehyde leads to unfolding of the RNA, and two components sedimenting at 11.0 S and at 14.3 S were observed. These values are in agreement with the results of

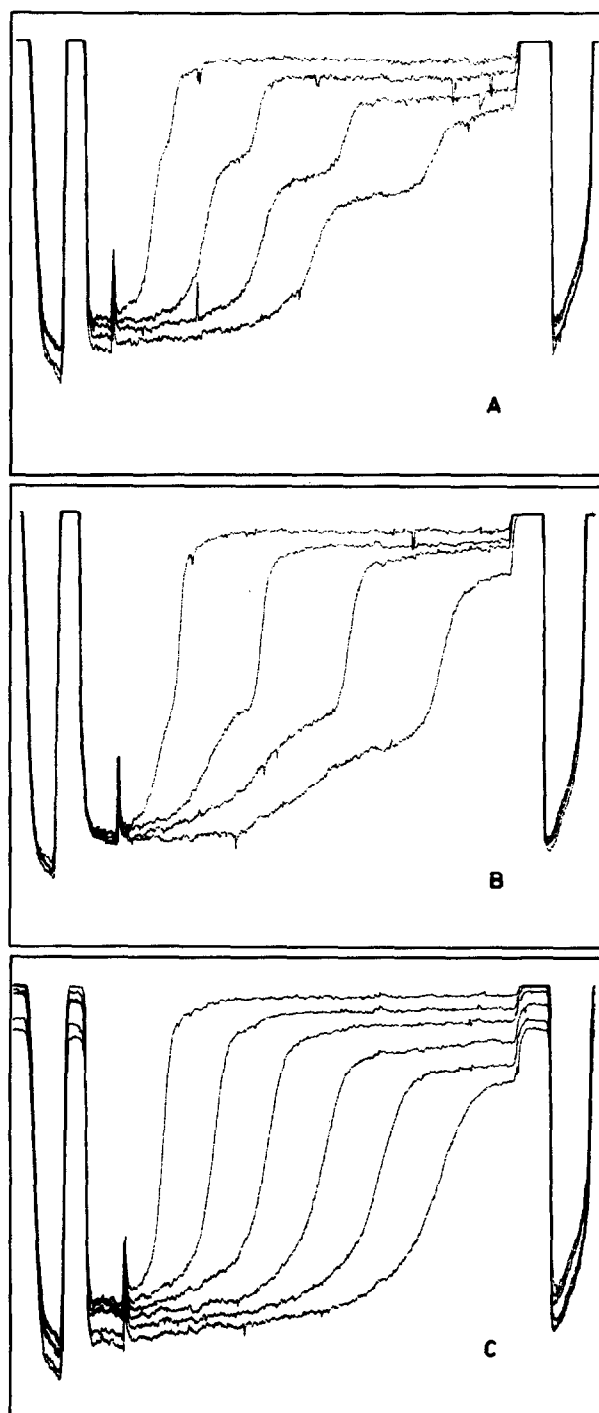


Fig. 2. Sedimentation patterns of the fast sedimenting components obtained in the reaction of formaldehyde with a mixture of 16 S and 23 S rRNAs (A), with purified 23 S rRNA (B), and with purified 16 S rRNA (C). Reactions with formaldehyde at pH 3.8 were carried out in two steps as described in the legend to fig. 1. Sedimentation at 20°C and 52,640 rpm. Desitometer tracings (displaced vertically) correspond to 8 min interval exposure. Sedimentation is from left to right.

Table 1  
Sedimentation coefficient of the fast components, obtained in the reaction with formaldehyde.

rRNA species	$S_{20,w}$	after reaction*
16 S + 23 S	38.2 S	24.9 S
23 S	37.9 S	(22.6 S)**
16 S		24.1 S

\* Calculated from the patterns shown in fig. 2.

\*\* Presumably derived from 23 S half molecules.

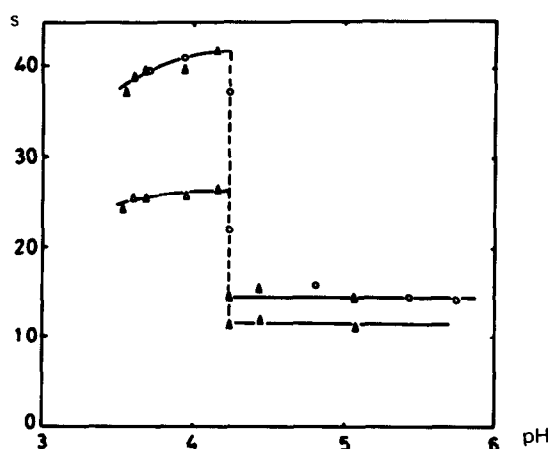


Fig. 1. Sedimentation coefficients of viral MS2 RNA (○) and of a mixture of 16 S (△) and 23 S (▲) rRNAs as a function of the pH of the reaction mixture.

Step I: 36  $\mu$ g RNA was heated for 20 min at 60°C in 7.7% formaldehyde at the pH shown (total volume 0.6 ml). The reaction was stopped by rapid cooling.

Step II: 0.4 ml of 0.2 M phosphate buffer (equimolar mixture) and  $2.5 \times 10^{-4}$  M EDTA was added, resulting in a final concentration of 0.08 M phosphate buffer,  $10^{-4}$  M EDTA and 1.5 M formaldehyde, pH 6.8.

Boedtke [14], who reported 10.9 S and 13.9 S for unfolded ribosomal RNA. At low salt concentration and when the pH of the reaction mixture was lowered from 5.0 to 3.6, two components sedimenting at 25 S and 38 S, instead of the unfolded forms, were found. The results are presented in fig. 1. The transition of unfolded molecules to fast sedimenting components occurred at pH 4.2, for the ribosomal RNAs and for the viral RNA.

A similar transition is observed when at pH 3.8 the NaCl concentration is reduced. Again, transition of the rRNA mixture was in the same range as the one

obtained with viral RNA, i.e. around  $6 \times 10^{-3}$  M NaCl (in the presence of  $10^{-4}$  M  $MgCl_2$ ). From these experiments we conclude that rRNA as well as viral MS2 RNA form dimers under the same conditions.

In the following experiment, we showed that the 38 S form was indeed derived solely from the 23 S monomer species, and vice versa the 25 S from the 16 S monomer. The two ribosomal RNAs were separated, and each preparation was reacted with formaldehyde in the presence of  $10^{-4}$  M  $MgCl_2$  at pH 3.8. The results (fig. 2 and table 1), show that each fast sedimenting component is indeed derived from one monomer species. The trailing, heterogeneous boundary present after reaction of purified 23 S rRNA with formaldehyde in dimer forming conditions is a consequence of "non-random" degradation of the 23 S in two half molecules, which in the absence of formaldehyde sediment at 16 S [15].

#### 4. Discussion

Several empirical relationships of the form  $S_{20,w} = k_s M.W.^{\alpha}$  have been proposed in order to estimate the molecular weight of RNAs on the basis of a comparison with reference RNA species. These functions are only valid for RNA molecules belonging to the same homologous series. Weak interactions in RNA molecules can be largely eliminated by reaction with formaldehyde. This results in unfolding of the RNA molecules, which sediment as a homologous series with exponent  $\alpha = 0.40$  [14, 16]. Unreacted *E. coli* ribosomal RNAs and bacteriophage MS2 RNA belong to another series with  $\alpha = 0.5$  [16]. The formaldehyde induced 38 S form of MS2 RNA, however, which is a dimer as shown by its sedimentation and intrinsic viscosity [11], also belongs to the same series. The sedimentation rates of the fast sedimenting ribosomal RNA derivatives, described here, fit the same equation, and on this basis we conclude that these are indeed homologous dimers.

Dimer formation is observed only when the polynucleotides have reached a critical degree of protonation (fig. 1). Furthermore a definite concentration of divalent cations are required [11]. The latter are displaced by excess monovalent cations, which results in unfolded monomers. Hyperchromicity studies indicate that also most of the base-pairs of the dimer species

have been opened as a result of adduct formation with formaldehyde. This is remarkable, considering that the sedimentation rate indicates a compact molecule. But this sedimentation rate is accounted for by a constrained conformation, even if the short, double stranded loops of the RNA have been disrupted. The dimer formation may perhaps be due to interaction between homologous, parallel-oriented stretches with high C or A content. Subsequently, this dimer is "fixed" by irreversible formation of formaldehyde-induced cross-links. The latter are presumably methylene bridges between purine bases [12, 13].

The results presented here indicate that the same dimer formation takes place under identical conditions for both ribosomal RNAs as for bacteriophage MS2 RNA. This means that specific recognition is not due to a very special feature, unique for the latter RNA. Even more important is the fact that in none of the reactions carried out on mixtures of both ribosomal RNAs was a complex between the 23 S and the 16 S species observed. Obviously, the recognition involved in dimer formation is highly sequence-specific. The non-competitive nature of dimer formation is somewhat analogous to the hybrid formation between the ribosomal RNAs and the corresponding DNA-cistrons, which was also highly specific for both of the two ribosomal RNAs [17].

#### Acknowledgement

This research was supported by grants from the Fonds voor Kollektief Fundamenteel Onderzoek

(No. 841). H.S. received a fellowship from the Instituut voor Wetenschappelijk Onderzoek in Nijverheid en Landbouw.

#### References

- [1] J.Marcot-Queiroz and R.Monier, *J. Mol. Biol.* 14 (1965) 490.
- [2] P.B.Moore and K.A.sano, *J. Mol. Biol.* 18 (1966) 21.
- [3] D.D.Henley, T.Lindahl and J.R.Fresco, *Proc. Natl. Acad. Sci. U.S.* 55 (1966) 191.
- [4] D.B.Millar and M.Mackenzie, *Biochem. Biophys. Res. Commun.* 23 (1966) 804.
- [5] J.H.Strauss and R.L.Sinsheimer, *J. Mol. Biol.* 7 (1963) 43.
- [6] E.O.Akinrimisi, C.Sander and P.O.P.Ts'O, *Biochemistry* 2 (1963) 340.
- [7] A.Rich, D.R.Davies, F.H.C.Crick and J.D.Watson, *J. Mol. Biol.* 3 (1961) 71.
- [8] A.Adams and H.G.Zachau, *European J. Biochem.* 5 (1968) 556.
- [9] H.G.Zachau, *European J. Biochem.* 5 (1968) 559.
- [10] W.Fiers, *Virology* 33 (1967) 413.
- [11] H.Slegers and W.Fiers, in press.
- [12] M.Ya.Feldman, *Biochim. Biophys. Acta* 149 (1967) 20.
- [13] V.D.Axelrod, M.Ya.Feldman, I.I.Chuguev and A.A.Bayev, *Biochim. Biophys. Acta* 186 (1969) 33.
- [14] H.Boetker, *J. Mol. Biol.* 35 (1968) 61.
- [15] W.Möller and H.Boetker, in: *Acids ribonucléiques et polyphosphates*, Centre National de la recherche scientifique (Paris, 1962) p.99.
- [16] M.L.Fenwick, *Biochem. J.* 107 (1968) 851.
- [17] S.A.Yankosky and S.Spiegelman, *Proc. Natl. Acad. Sci. U.S.* 49 (1963) 538.